

*Sub
B1
A1*
~~said embryonic cells selected from embryonic stem cells, fertilized egg cells, and cells of 2-cell embryos;~~

ii) ~~[an] a chemical agent capable of producing at least one modification in said gene of interest in at least one of said [target] embryonic cells and at least one modification in one or more additional genes;~~

b) ~~treating said [target] embryonic cells with said agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising cells having an unmodified gene of interest and cells having a modified gene of interest; and~~

c) ~~isolating said cells having a modified gene of interest.~~

*Sub
B2
A2*
3. (Once amended) The method of Claim [2] 1, further comprising [e) manipulating said cells] ~~d) placing at least one of said cells having a modified gene of interest into an environment under conditions so as to generate an organism comprising said modification in said gene of interest.~~

A3
9. (Once amended) The method of Claim 1, wherein said [target cell is] ~~embryonic cells are derived from [an organism selected from the group consisting of] a non-human animal[, plant, protist, fungus, bacterium, and virus].~~

A4
13. (Once amended) The method of Claim 1, wherein said [target] ~~embryonic~~ cell is ~~[an] a mouse~~ embryonic stem cell

14. (Once amended) The method of Claim 1, wherein said agent is selected from the group consisting of *N*-ethyl-*N*-nitrosurea, methylnitrosourea, procarbazine hydrochloride, triethylene melamine, acrylamide monomer, chlorambucil, melphalan, cyclophosphamide, diethyl sulfate, ethyl methane sulfonate, methyl methane sulfonate, 6-mercaptopurine, mitomycin-C, procarbazine, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, $^3\text{H}_2\text{O}$, and urethane[, ultraviolet light, X-ray radiation, and gamma-radiation].

Sub B3

continue A4

15. (Once amended) A method of producing an allelic series of [modification] modifications in a gene of interest contained in a cell, comprising:

- a) providing:
 - i) [a plurality] an *in vitro* culture of target cells [capable of being cultured] comprising isolated embryonic cells containing a gene of interest, said embryonic cells selected from embryonic stem cells, fertilized egg cells, and cells of 2-cell embryos;
 - ii) [an] a chemical agent capable of producing at least one modification in said gene of interest in at least one of said [target] embryonic cells;
- b) treating said [target] embryonic cells with said agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising cells having an unmodified gene of interest, cells having a first modification in said gene of interest, and cells having a second modification in said gene of interest; and
- c) isolating said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest, thereby producing an allelic series of [modification] modifications in said gene of interest in the isolated cells.

Sub B4

A5

17. (Once amended) The method of Claim [16] 15, further comprising [e) manipulating cells] d) placing at least one cell selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest into an environment under conditions so as to generate an organism comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.

A6

23. (Once amended) The method of Claim 15, wherein said [target cell is] embryonic cells are derived from [an organism selected from the group consisting of] a non-human animal[, plant, protist, fungus, bacterium, and virus].

A7 27. (Once amended) The method of Claim 15, wherein said [target] embryonic cell is [an] a mouse embryonic stem cell.

28. (Once amended) The method of Claim 15, wherein said agent is selected from the group consisting of *N*-ethyl-*N*-nitrosourea, methylnitrosourea, procarbazine hydrochloride, triethylene melamine, acrylamide monomer, chlorambucil, melphalan, cyclophosphamide, diethyl sulfate, ethyl methane sulfonate, methyl methane sulfonate, 6-mercaptopurine, mitomycin-C, procarbazine, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, $^3\text{H}_2\text{O}$, and urethane[, ultraviolet light, X-ray radiation, and gamma-radiation].

Please add the following claims:

A8 29 (New) A method of producing a modification in a gene of interest contained in a cell, comprising:

- a) providing:
 - i) an *in vitro* culture of target cells comprising isolated embryonic cells containing a gene of interest, said embryonic cells selected from protocorm-like body cells, and callus cells.;
 - ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells and at least one modification in one or more additional genes;
- b) treating said embryonic cells with said agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising cells having an unmodified gene of interest and cells having a modified gene of interest;
- c) isolating said cells having a modified gene of interest; and
- d) placing at least one of said cells having a modified gene of interest into an environment under conditions so as to generate an organism comprising said modification in said gene of interest.

30. (New) A method of producing a modification in a gene of interest contained in a mouse cell, comprising:

a) providing:

- i) an *in vitro* culture of isolated mouse embryonic stem cells containing a gene of interest;
- ii) a chemical agent capable of producing at least one modification in said gene of interest in at least one of said mouse embryonic stem cells and at least one modification in one or more additional genes;

b) treating said mouse embryonic stem cells with said agent under conditions such that a mixture of embryonic stem cells is produced, said mixture of embryonic stem cells comprising cells having an unmodified gene of interest and cells having a modified gene of interest;

c) isolating said cells having a modified gene of interest;

d) comparing the nucleotide sequence of said gene of interest in said cells having a modified gene of interest with the nucleotide sequence of said gene of interest in said cells having an unmodified gene of interest; and

e) manipulating said cells having a modified gene of interest to generate an organism comprising said modification in said gene of interest, wherein said manipulating comprises:

- i) introducing said cells having said modified gene of interest into a mouse embryo to produce a treated embryo;
- ii) introducing said treated embryo into a pseudopregnant mouse; and
- iii) permitting said pseudopregnant mouse to deliver at least one offspring comprising said modified gene of interest.

31. (New) The method of Claim 3, wherein said organism is chimeric.

32. (New) A method of producing an allelic series of modifications in a gene of interest contained in a cell, comprising:

a) providing:

1) *an in vitro culture of target cells comprising isolated embryonic cells containing a gene of interest, said embryonic cells selected from protocorm-like body cells, and callus cells;*

ii) *a chemical agent capable of producing at least one modification in said gene of interest in at least one of said embryonic cells;*

b) *treating said embryonic cells with said agent under conditions such that a mixture of embryonic cells is produced, said mixture of embryonic cells comprising cells having an unmodified gene of interest, cells having a first modification in said gene of interest, and cells having a second modification in said gene of interest;*

c) *isolating said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated cells; and*

d) *placing at least one cell selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest into an environment under conditions so as to generate an organism comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest.*

33. (New) A method of producing an allelic series of modifications in a gene of interest contained in a mouse cell, comprising:

a) *providing:*

i) *an in vitro culture of mouse embryonic stem cells;*

ii) *a chemical agent capable of producing at least one modification in said gene of interest in at least one of said mouse embryonic stem cells;*

b) *treating said mouse embryonic stem cells with said agent under conditions such that a mixture of embryonic stem cells is produced, said mixture of embryonic stem cells comprising cells having an unmodified gene of interest, cells having a first modification in said gene of interest, and cells having a second modification in said gene of interest;*

Continued
As

c) isolating said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest, thereby producing an allelic series of modifications in said gene of interest in the isolated cells;

d) comparing the nucleotide sequence of said gene of interest in said cells having an unmodified gene of interest with the nucleotide sequence of said gene of interest in cells selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest; and

e) manipulating cells selected from the group consisting of said cells having a first modification in said gene of interest and said cells having a second modification in said gene of interest to generate an organism comprising a modification selected from the group consisting of said first modification in said gene of interest and said second modification in said gene of interest, wherein said manipulating comprises:

i) introducing said cells having said first modification in said gene of interest and said cells having said second modification of said gene of interest into a mouse embryo to produce a treated embryo;

ii) introducing said treated embryo into a pseudopregnant mouse; and

iii) permitting said pseudopregnant mouse to deliver at least one offspring comprising said first modification in said gene of interest or said second modification in said gene of interest.

*Sub
B*

34. (New) The method of Claim 17, wherein said organism is chimeric.

REMARKS

Status of the Application

Claims 1-28 are pending in the present application. Applicants hereby confirm their provisional election without traverse of Claims 1-28.¹

¹ See Office Action, page 2, final full paragraph.